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Pharmacokinetics of Lamivudine and Lamivudine-Triphosphate after Administration of 300 Milligrams and 150 Milligrams Once Daily to Healthy Volunteers: Results of the ENCORE 2 Study

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There is interest in evaluating the efficacy of lower doses of certain antiretrovirals for clinical care. We determined here the bioequivalence of plasma lamivudine (3TC) and intracellular 3TC-triphosphate (3TC-TP) concentrations after the administration of two different doses. ENCORE 2 was a randomized crossover study. Subjects received 3TC at 300 and 150 mg once daily for 10 days (arm 1; $n = 13$) or vice versa (arm 2; $n = 11$), separated by a 10-day washout. Pharmacokinetic (PK) profiles (0 to 24 h) were assessed on days 10 and 30. Plasma 3TC and 3TC-TP levels in peripheral blood mononuclear cells were quantified by high-performance liquid chromatography-tandem mass spectrometry. Within-subject changes in PK parameters (the area under the concentration-time curve from 0 to 24 h [$AUC_{0\text{-}24}$], the trough concentration of drug in plasma at 24 h [C_{24}], and the maximum concentration of drug in plasma [C_{max}]) were evaluated by determining the geometric mean ratios (GMRs) adjusted for study arm, period, and intra-individual variation. Regimens were considered bioequivalent if the 90% confidence interval (90% CI) fell within the range of 0.8 to 1.25. A total of 24 subjects completed the study. The GM (90% CI) 3TC $AUC_{0\text{-}24}$), expressed as ng·h/ml, for the 300- and 150-mg doses were 8,354 (7,609 to 9,172) and 4,773 (4,408 to 5,169), respectively. Bioequivalence in 3TC PK following the administration of 300 and 150 mg was not demonstrated: the GMRs for $AUC_{0\text{-}24}$, C_{24}, and C_{max} were 0.57 (0.55 to 0.60), 0.63 (0.59 to 0.67), and 0.56 (0.53 to 0.60), respectively. The GM (90% CI) 3TC-TP $AUC_{0\text{-}24}$ values (pmol·h/10^6 cells) for the 300- and 150-mg doses were 59.5 (51.8 to 68.3) and 44.0 (38.0 to 51.0), respectively. Bioequivalence in 3TC-TP PK following the administration of 300 and 150 mg was not demonstrated: the GMRs for $AUC_{0\text{-}24}$, C_{24}, and C_{max} were 0.73 (0.64 to 0.83), 0.82 (0.68 to 0.99), and 0.70 (0.61 to 0.82), respectively. We found that 3TC at 150 mg is not bioequivalent to the standard regimen of 300 mg, indicating that saturation of cytosine phosphorylation pathways is not achieved at a dose of 150 mg.

Lamivudine (3TC), a commonly used nucleoside reverse transcriptase inhibitor (NRTI), is an inactive prodrug that requires transport into the cell and stepwise phosphorylation by intracellular kinases to produce its active triphosphate form-lamivudine triphosphate (3TC-TP) (5). The active triphosphate competes with the corresponding endogenous nucleoside triphosphate, dCTP, for binding to the viral reverse transcriptase. Once incorporated into viral DNA, chain termination results due to absence of a 3′-hydroxy group to which 3′-5′-phosphodiester linkages are normally made (12).

Studies conducted in HIV-infected patients have failed to establish clear pharmacokinetic-pharmacodynamic relationships between the plasma 3TC concentrations and antiviral efficacy and safety, whereas the concentrations of its intracellular triphosphate, 3TC-TP, have been shown to be the critical parameter to predict efficacy and toxicity *in vivo* (1, 8).

The active nucleoside triphosphates are trapped intracellularly due to the presence of ionic phosphate groups and this confers the long intracellular half-lives compared to the respective parent compounds in plasma. Since 3TC-TP is characterized by a long intracellular half-life (ca. 15 to 16 h), active triphosphate concentrations persist in cells after plasma 3TC (half-life, ~5 h) concentrations have decreased, thus enabling less frequent dosing (18).

3TC is currently approved at a dose of 150 mg twice daily (BID) or 300 mg once daily (QD). During the clinical development of 3TC, no clear correlation between its dose and reductions in HIV-RNA or other surrogate markers in HIV-infected individuals were demonstrated; including in the NUCB2001 trial, at doses of 35 to 1,400 mg/day (27). The NUCA3001 (treatment naive) and NUCA3002 (treatment experienced) trials reported no differences in reduction in HIV-RNA between patients taking 3TC at 300 mg BID versus 150 mg BID (4, 7) and, in a pharmacokinetic substudy of NUCA3001, only small elevations in 3TC triphosphate concentrations were found in patients receiving the 300-mg BID regimen (18). Similarly, a pharmacokinetic study found intracellular 3TC-TP exposures to be bioequivalent at 150-mg BID and 300-mg QD doses (28), and in a phase III (treatment naive) trial, no differences in efficacy were reported between these regimens (6). The combined data from dose-ranging and pharmacokinetic studies suggest that saturation of intracellular phosphorylation pathways may be occurring at higher 3TC doses and, in turn, provide an incentive to evaluate lower unit doses of 3TC.

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This study aimed to evaluate (i) the plasma and intracellular pharmacokinetics of 3TC and its active triphosphate 3TC-TP and (ii) the safety and tolerability of 3TC following administration of 3TC in 300-mg and 150-mg QD doses to HIV-negative healthy volunteers.

(The results of this study were presented in part at the 12th International Workshop on Clinical Pharmacology of HIV Therapy, Miami, FL, 13 to 15 April 2011.)

MATERIALS AND METHODS

Subjects. Male and female (nonpregnant, nonlactating) subjects were eligible for enrolment if they provided written informed consent and met the following criteria: age between 18 and 65 years and body mass index (BMI) 18 to 35 kg/m^2. Subjects were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screen to HIV or hepatitis B or C; current or recent (within 3 months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within 3 months of first dose of study drug; use of any other drugs, including over-the-counter medications and herbal preparations, within 2 weeks prior to first dose of study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

Study design. This was a 31-day, open-label, prospective, two-arm crossover pharmacokinetic study conducted at the Pharmacokinetic Unit of the St. Stephen's Centre, Chelsea and Westminster Hospital, London, England. The study protocol was reviewed and approved by the Riverside Research Ethics Committee (United Kingdom). All subjects provided written informed consent and the trial was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and applicable regulatory requirements (EudraCT 2009-011844-20).

At screening, subjects had a clinical assessment and routine laboratory investigations performed. The safety and tolerability of study medications were evaluated throughout the study using the NIAID Division of AIDS table for grading the severity of adult and pediatric adverse events to characterize abnormal findings (published December 2004), vital signs, physical examinations, clinical laboratory investigations, and serial ECGs.

Following successful screening, subjects were randomized to (i) arm 1 and administered 3TC at 300 mg QD for 10 days (days 1 to 10), followed by a 10-day washout period (days 11 to 20), followed by 3TC at 150 mg QD for 10 days (days 21 to 30) or (ii) arm 2 and administered 3TC at 150 mg QD for 10 days (days 1 to 10), followed by a 10-day washout period (days 11 to 20), followed by 3TC at 300 mg QD for 10 days (days 21 to 30).

Subjects were administered study drugs in the morning within 15 min of completion of a standardized breakfast, along with 240 ml of water. Subjects received a standardized lunch at approximately 4 h after dosing. Pharmacokinetic sampling was performed on days 10 and 11 and days 30 and 31. Serial blood specimens for intracellular 3TC-TP and plasma 3TC were taken at the following time points: predose (0 h) and 1, 2, 4, 8, 12, and 24 h postdose.

PBMC isolation. Blood samples for determination of intracellular pharmacokinetic measurements in peripheral blood mononuclear cells (PBMCs) were collected in two 8-ml cell preparation tubes (CPT; Becton Dickinson Vacutainer) per time point. Tubes were mixed gently by inversion until centrifugation at room temperature (20°C) for 20 min at 1,600 relative centrifugal force (RCF). After centrifugation, the upper layers of plasma and PBMCs were gently mixed and transferred from both CPT to a single graduated 50-ml conical tube. Isotonic saline (0.9%) was added to bring the volume to exactly 30 ml. The sample was then gently mixed by inversion, and a 40-μl aliquot was taken for initial cell counting (Digital Bio Adam Microchip Automatic Cell Counter; NanoEnTek, Inc., Seoul, Korea). The count was multiplied by 30 to calculate the total number of cells in 30 ml and recorded. The samples were centrifuged for 15 min at 400 RCF to pellet the cells, and the supernatant was removed. The cell pellet was suspended in 1 ml of 70% methanol, vortexed (3 to 5 min) to ensure cell lysis, and stored at −80°C. Samples were shipped on dry ice to the Liverpool Biomedical Research Centre Bioanalytical Facility (GCLP accredited) for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Analytical methods. (i) Quantification of lamivudine from plasma. Plasma 3TC concentrations were quantified by a validated solid-phase extraction (SPE) method coupled with reversed-phase high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS).

Plasma samples (300 μl) were prepared in formic acid (300 μl; 0.02%) containing an internal standard, 2-chloroadenosine (Cl-A; 5 μg/ml; 20 μl), and loaded onto a 100-mg SPE BondElut C_{18} column (Varian, Inc., Netherlands). Columns were conditioned with methanol (1 ml) and 0.02% formic acid (1 ml; pH 3). Samples (400 μl) were loaded onto the column and rinsed with 0.02% formic acid (200 μl). Analytes were eluted with methanol (100 μl), evaporated to dryness, reconstituted in 100 μl of mobile phase, and injected (10 μl) onto the HPLC column.

Chromatography was performed on a Synergi polar C_{18} column (4 μm, 150 mm by 2.0 mm; Phenomenex, Cheshire, United Kingdom). Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile (ACN). Initial conditions consisted of 99% mobile phase A, increasing in organic content to 40% mobile phase B in 0.7 min maintained over 1.3 min and equilibrated back to the initial conditions over a total run time of 6 min. The flow rate was 400 μl/min. The triple-quadrupole mass spectrometer (TSQ Quantum Access; Thermo Electron Corp., Hemel Hempstead, United Kingdom) was operated in positive ionization mode, and detection and quantification was performed using multiple reaction monitoring (MRM).

The assay was validated over a calibration range of 6.9 to 5,137 ng/ml. The interday precision (percent coefficient of variation [CV%]) based on quality control (QC) samples was between 3.9 and 6.8%, and accuracy (percent bias) was ±11.6%. The percentage recovery was >90%.

(ii) Quantification of intracellular lamivudine-triphosphate from PBMC. Intracellular 3TC-TP concentrations were quantified from human PBMC using a validated ion-pair reversed-phase HPLC-MS/MS method. 3TC-TP [(−)-β-L-2′,3′-dideoxy-3′-thiacytidine triphosphate] was purchased from Moravek Biochemicals (Brea, CA), and an internal standard 2-chloroadenosine 5′-triphosphate (Cl-ATP) was purchased from Sigma-Aldrich, United Kingdom.

Blank human PBMCs were isolated from drug-free human buffy coat via density gradient centrifugation. The cells were prepared in 70% methanol and dispatched in fractions of approximately 2×10^6 cells. Calibrators/QC were prepared from master stock solutions (1 mg/ml in ultrapure water). A master stock solution of Cl-ATP was prepared identically in water. All stock solutions were stored at −80°C. Stock solutions were further diluted with PBMC lysate (2×10^6 cells in 70% methanol) to yield working solutions containing 3TC-TP, which were then dispensed (1 ml) into labeled screw-cap tubes and stored at −80°C until use. A working internal standard solution was prepared by dilution of Cl-ATP to 4 μg/ml and stored at −40°C.

On the day of analysis, 1 ml of LC-MS-grade ACN was added to the calibrators/QC and clinical samples. Samples were vortexed and centrifuged (13,000 rpm, 4°C), and the supernatant was transferred to fresh glass tubes. Cl-ATP (20 μl; 4 μg/ml) was added, and the samples were evaporated to dryness with a nitrogen stream evaporator (20°C). The samples were then resuspended in 100 μl of 5 mM ammonium formate and 50 mM *N*,*N*-dimethylhexylamine (DMHA) at 1:1 (vol/vol). Sufficient sample was transferred into autosampler vials ready for injection (25 μl) onto the HPLC column.

Analytes were eluted using a Fortis C_{18} column (3 μm, 150 by 2.1 mm; Fortis Technologies, Ltd., Cheshire, United Kingdom). Mobile phase A (pH ~8.6) consisted of DMHA (5 mM) and 10 mM ammonium formate–water (1:1 [vol/vol]), and mobile phase B consisted of DMHA (5 mM) and 10 mM ammonium formate–ACN (1:1 [vol/vol]). Initial conditions consisted of 90% mobile phase A, increasing in organic content to 50% mo-

TABLE 1 Geometric mean plasma 3TC pharmacokinetic parameters during 3TC 300-mg and 150-mg regimens[a]

Parameter	QD dose (mg)	Geometric mean (90% CI)	Full mixed model: Dose GMR (90% CI)	Full mixed model: Dose *P*	Full mixed model: Arm *P*	Full mixed model: Period *P*	Basic mixed model: Dose GMR (90% CI)
$AUC_{0\text{-}24}$ (ng · h/ml)	150	4,773 (4,408–5,169)	0.57 (0.55–0.60)	**<0.001**	**0.002**	0.277	0.57 (0.56–0.59)
	300	8,354 (7,609–9,172)					
C_{24} (ng/ml)	150	38.1 (34.0–42.7)	0.63 (0.59–0.67)	**<0.001**	0.105	0.955	0.63 (0.59–0.67)
	300	60.8 (53.4–69.2)					
C_0 (ng/ml)	150	39.4 (34.1–45.5)	0.64 (0.58–0.70)	**<0.001**	**0.040**	0.416	0.64 (0.59–0.71)
	300	61.3 (52.6–71.3)					
C_{max} (ng/ml)	150	757 (688–833)	0.56 (0.53–0.60)	**<0.001**	**0.019**	0.558	0.56 (0.53–0.60)
	300	1,344 (1,247–1,448)					
$t_{1/2}$ (h)	150	5.91 (5.63–6.19)	1.03 (0.99–1.08)	0.186	0.315	0.807	1.12 (0.96–1.32)
	300	5.71 (5.50–5.94)					
T_{max} (h)	150	1.73 (1.50–2.00)	1.15 (1.00–1.32)	0.107	0.851	0.004	1.12 (0.96–1.32)
	300	1.54 (1.34–1.77)					

[a] GMRs were calculated using 3TC at 300 mg as the referent group. A full mixed model (fitting dose, study arm, and treatment period with adjustment for crossover) and a basic mixed model (fitting dose with adjustment for crossover) were utilized. Statistically significant *P* values are indicated in boldface.

bile phase B in 11.2 min (maintained for 2 min). The column was equilibrated back to the initial conditions over a total run time of 19 min. The flow rate was set at 350 μl/min. The triple-quadrupole mass spectrometer (TSQ Quantum Access) was operated in negative ionization mode, and detection and quantification was performed using MRM.

The assay was validated over a calibration range of 1.5 to 100 ng/sample (ca. 2- to 200 pmol). The interday precision of QC samples was between 7.7 to 11%, and the % bias was ±2.0% (2 to 85 ng/sample). The percentage recovery was >80%.

(iii) Pharmacokinetic and statistical analysis. The pharmacokinetic parameters for the 3TC and 3TC-TP maximum concentration (C_{max}), the time to C_{max} (T_{max}), and the trough concentrations at 0 and 24 h (C_0 and C_{24}) were derived from the concentration-time profiles. The area under the curve concentration-time curve from 0 to 24 h ($AUC_{0\text{-}24}$) and the elimination half-life ($t_{1/2}$) were calculated using WinNonlin software (version 6.1; Pharsight Corp., Mountain View, CA). Pharmacokinetic parameters were expressed as unadjusted geometric means (GM) and associated 90% confidence intervals (90% CI). Inter-individual variability in plasma concentrations was assessed by measuring the coefficient of variation [CV = (standard deviation/mean) × 100].

Within-subject changes of drug concentrations were assessed by geometric mean ratios (GMRs) and associated 90% CI (using a 3TC 300-mg QD dose as the reference group) after adjustment for the study arm, treatment period (day 10 or 30), and crossover design (intra-individual variation) using linear mixed-effects modeling (SAS; version 9.1). A basic mixed model was also fitted (correcting only for crossover design) and used as a reference in determining the clinical importance of differences between study arms or treatment period. The results were considered statistically significant at $\alpha < 0.10$ or if the 90% CI of the GMR excluded 1. No adjustments were made for multiple comparisons. 3TC doses were considered bioequivalent if the 90% CI for the GMR fell within the acceptance range of 0.8 to 1.25.

RESULTS

Demographic and clinical characteristics. Twenty-four subjects (13 [54%] female) completed the study, which included 13 subjects in arm 1 (10 female) and 11 subjects (3 female) in arm 2. The median (range) age, weight, and body mass index (BMI) were 34 (27 to 57) years, 72 (49 to 110) kg, and 24 (20 to 35) kg/m^2, respectively. Sixteen subjects were Caucasian, four were black, one was of Asian origin, and three defined themselves as "other" (not specified). The study drugs were well tolerated, and no grade 3 or 4 adverse events were reported. Lipid, total cholesterol, and triglyceride levels were normal at screening and at days 37 to 44 follow-up.

Plasma lamivudine pharmacokinetics. The plasma 3TC pharmacokinetic parameters for all 24 study participants (arm 1 and arm 2) are presented in Table 1, and the pharmacokinetic profiles over 24 h are illustrated in Fig. 1, respectively. The elimination half-life did not differ between the two doses. Bioequivalence in plasma 3TC pharmacokinetic parameters, following 300-mg and 150-mg QD regimens, was not demonstrated as the GMR (90% CI) were 0.57 (0.55 to 0.60) for AUC_{24}, 0.63 (0.59 to 0.67) for C_{24}, and 0.56 (0.53 to 0.60) for C_{max}.

Statistically significant differences in 3TC plasma concentrations were observed between the two study arms (data not shown), in which participants in arm 1 had, on average, higher 3TC plasma exposures than participants in arm 2, independent of the dosing regimen or treatment period. However, despite these differences, the study arm had very little influence on the overall effect of dose upon 3TC pharmacokinetic outcomes. When we compared the full mixed model to the basic mixed model (Table 1), almost identical GMR estimates of the effect of dose were evident, independent of the study arm.

Intracellular lamivudine triphosphate pharmacokinetics. Intracellular 3TC-TP pharmacokinetic parameters are presented in Table 2, and the pharmacokinetic profiles are reported in Fig. 2.

The GM (90% CI) intracellular 3TC-TP AUC_{24} (pmol.h/10^6 cells), C_{24}, and C_{max} (pmol/10^6 cells) for the 300-mg dose were 59.5 (51.8 to 68.3), 1.49 (1.19 to 1.86), and 4.10 (3.59 to 4.69), respectively. For the 150-mg dose, they were 44.0 (38.0 to 51.0), 1.23 (1.00 to 1.52), and 2.95 (2.47 to 3.51), respectively. Bioequivalence in intracellular 3TC-TP pharmacokinetic parameters,

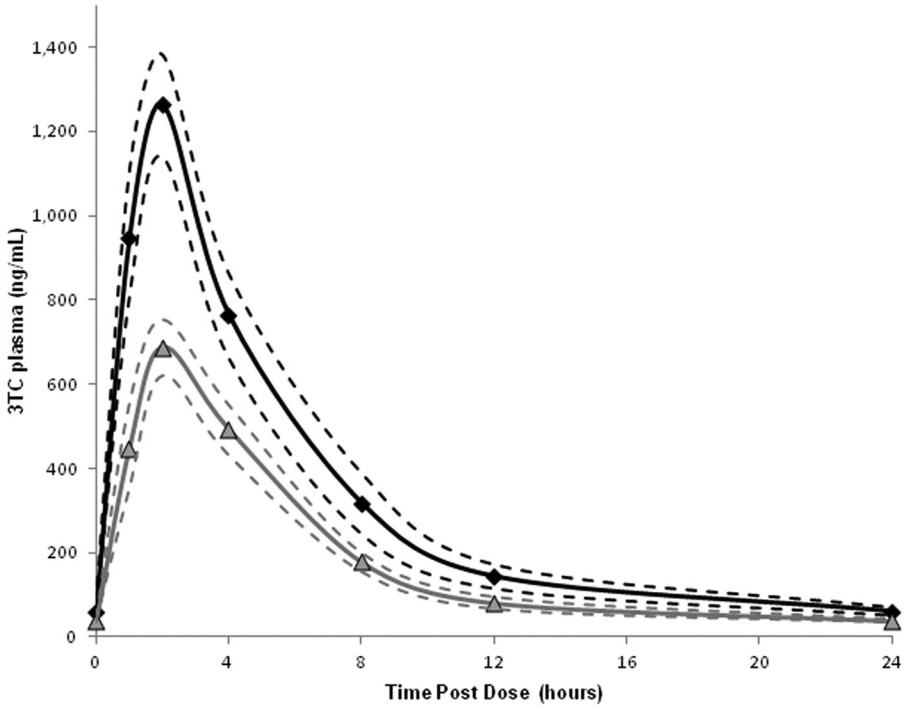

FIG 1 Steady-state plasma 3TC geometric mean (90% CI) concentrations over 24 h during 300-mg and 150-mg intakes ($n = 24$). Solid black diamonds, 3TC 300-mg QD regimen; gray triangles, 3TC 150-mg QD regimen; dashed lines, 90% CI.

following 300-mg and 150-mg QD regimens, was not demonstrated since the GMRs (90% CI) were 0.73 (0.64 to 0.83) for AUC_{24}, 0.82 (0.68 to 0.99) for C_{24}, and 0.70 (0.61 to 0.82) for C_{max}.

Although subjects received timed and witnessed drug doses after a standardized meal on the pharmacokinetic study days, the interpatient variability of 3TC-TP AUC_{24}, C_{24}, and C_{max} values ranged between 36 and 62%. Plasma 3TC and intracellular 3TC-TP concentrations ($n = 24$) were not significantly correlated.

A significant effect of treatment period on intracellular drug exposure was observed. 3TC-TP exposures were, on average, higher at day 30 than at day 10, independent of dose and of study arm ($P = 0.008$). Similar statistically significant differences were also observed for C_0 ($P = 0.007$) and C_{max} ($P = 0.008$). However, despite being a statistically significant predictor, treatment period had very little impact on the observed relationship between dose and pharmacokinetic outcomes. When we compared the full mixed model to the basic mixed model, independent of adjustment for differences in treatment period, we obtained comparable GMR estimates of the dose effect.

Interestingly, when data were stratified according to study arm,

TABLE 2 Intracellular 3TC-TP pharmacokinetic parameters during 3TC 300-mg and 150-mg regimens[a]

Parameter	QD dose (mg)	Geometric mean (90% CI)	Full mixed model				Basic mixed model
			Dose		Arm	Period	Dose
			GMR (90% CI)	*P*	*P*	*P*	GMR (90% CI)
$AUC_{0\text{-}24}$ (pmol · h/10^6 cells)	150	44.0 (38.0–51.0)	0.73 (0.64–0.83	**<0.001**	0.637	**0.008**	0.74 (0.64–0.86)
	300	59.5 (51.8–68.3)					
C_{24} (pmol/10^6 cells)	150	1.23 (1.00–1.52)	0.82 (0.68–0.99)	**0.083**	0.404	0.246	0.83 (0.68–1.01)
	300	1.49 (1.19–1.86)					
C_0 (pmol/10^6 cells)	150	1.41 (1.14–1.74)	0.74 (0.61–0.90)	**0.012**	0.796	**0.007**	0.76 (0.61–0.95)
	300	1.84 (1.52–2.22)					
C_{max} (pmol/10^6 cells)	150	2.95 (2.47–3.51)	0.70 (0.61–0.82)	**<0.001**	0.981	**0.008**	0.72 (0.61–0.85)
	300	4.10 (3.59–4.69)					
T_{max} (h)	150	5.11 (3.80–6.87)	1.01 (0.72–1.43)	0.944	**0.013**	**0.027**	0.98 (0.68–1.41)
	300	5.24 (3.98–6.90)					

[a] GMRs were calculated using 3TC at 300 mg as the referent group. A full mixed model (fitting dose, study arm, and treatment period with adjustment for crossover) and a basic mixed model (fitting dose with adjustment for crossover) were utilized. Statistically significant *P* values are indicated in boldface.

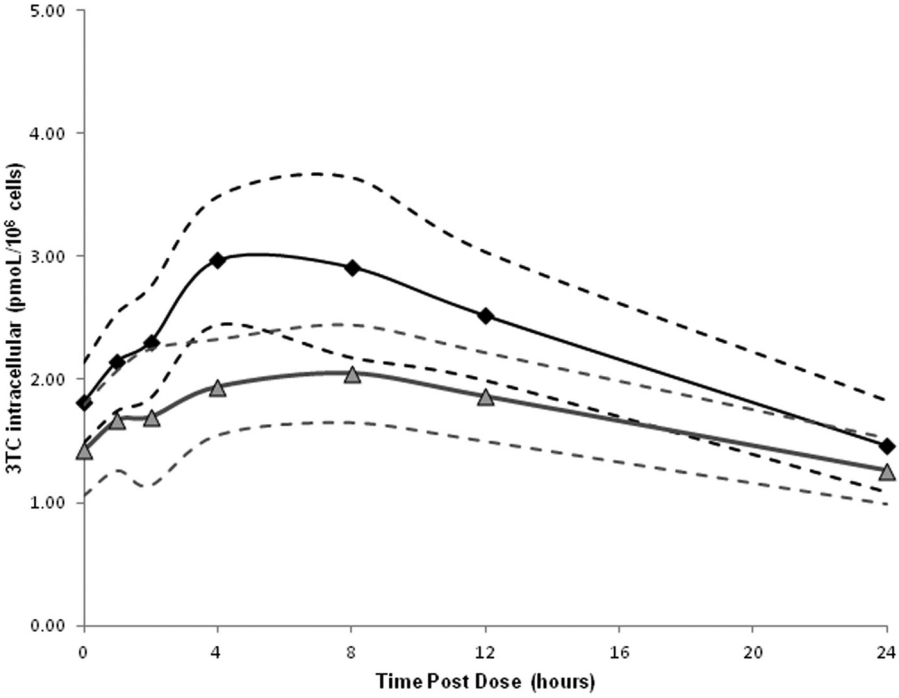

FIG 2 Steady-state intracellular 3TC-TP geometric mean (90% CI) concentrations over 24 h during 300-mg and 150-mg intakes ($n = 24$). Solid black diamonds, 3TC 300-mg QD regimen; gray triangles, 3TC 150-mg QD regimen; dashed lines, 90% CI.

no significant differences were observed in 3TC-TP exposures (AUC_{24}) between the 3TC 300-mg and 150-mg dosing in arm 1, when 300-mg dosing preceded 150-mg dosing (the AUC_{24} values were 52.3 and 47.5 pmol·h/10^6 cells, respectively [$P = 0.374$]), whereas a significant difference ($P < 0.001$) was seen when we compared the 3TC-TP AUC_{24} in arm 2 when 150-mg dosing preceded 300-mg dosing (the AUC_{24} values were 40.5 and 69.2 pmol·h/10^6 cells, respectively).

DISCUSSION

This study investigated the steady-state pharmacokinetics of 3TC when administered at 150 mg and 300 mg QD to healthy HIV-negative volunteers. Both plasma 3TC concentrations and intracellular 3TC-TP concentrations were measured. A study population consisting of healthy subjects was deemed appropriate, since there are no reports of differences in 3TC pharmacokinetics between healthy and HIV-infected populations (19, 20, 28). Importantly, it is not possible to conduct a study with monotherapy lamivudine in HIV-infected subjects because of the high likelihood of HIV drug resistance in this setting.

Administration of different 3TC doses resulted in significantly lower plasma 3TC and intracellular 3TC-TP pharmacokinetic parameters during intake of 150 mg QD. Consequently, bioequivalence between low dose 3TC at 150 mg QD and the standard 300-mg QD regimen was not achieved. Both doses were well tolerated, and no unexpected or serious adverse events related to the study medication were reported. The steady-state plasma 3TC and intracellular 3TC-TP pharmacokinetic parameters observed in the present study with 300 mg QD were comparable to that previously reported in healthy volunteers (2, 3, 28) and HIV-infected patients (2, 9, 14, 17, 18, 21) receiving 3TC doses at 300 mg QD or 150 mg BID. However, there was marked intersubject variation in 3TC-TP concentrations, both here and in previous studies (the CV% invariably exceeded 50%), and differences between study centers, with some reporting slightly higher 3TC-TP concentrations (6 to 11 pmol/10^6 cells) in HIV-infected patients (13, 21). Such variability in nucleoside triphosphate concentrations may be attributed to a number of interrelated factors that relate to the patient/host (immune status, cellular function, and genetic variation) intracellular pharmacokinetics (endogenous enzyme activity, competition with endogenous deoxynucleoside triphosphates, and concomitantly administered drugs) and differences in bioanalytical methods. For example, some centers analyze nucleoside triphosphates indirectly by separating the intracellular nucleotides using solid-phase extraction (SPE), followed by enzymatic dephosphorylation, desalting, and quantification by HPLC-MS/MS (16, 23). Others analyzed the nucleoside triphosphates directly via HPLC-MS/MS without dephosphorylation. However, due to the chemical similarity and high polarity of the endogenous and NRTI triphosphates, which cannot be easily retained via conventional reversed-phase chromatography, most methods have been developed using weak anion-exchange chromatography (24) or ion-pair reversed-phase chromatography (10, 22). There is currently no external QA scheme for nucleoside triphosphates.

Significant differences in plasma 3TC pharmacokinetics were observed between the two study arms. Given that arm 1 contained a higher proportion of females than arm 2 (76% versus 27%), differences in pharmacokinetic outcomes between the arms could potentially be gender related. This was confirmed through a secondary analysis in which gender was included into the full mixed model (data not shown). Including gender transferred much of the statistical significance of the difference in 3TC plasma AUC_{24} and C_{max} from study arm ($P = 0.06$ and $P = 0.38$ when gender is

included) to gender ($P = 0.06$ and $P = 0.002$). However, the present investigation was neither designed nor powered to properly investigate gender effects. For example, given that both 300-mg and 150-mg doses were taken by all 24 participants and within each treatment period, adjusting for gender has, by definition, no impact on the observed results for either dose or treatment period.

There is currently no evidence in the literature to suggest gender-related differences in 3TC pharmacokinetics when normalized for body weight (26). Gender-related differences in intracellular 3TC-TP pharmacokinetics have been reported previously, with most, but by no means all (2), studies observing a trend toward elevated TP concentrations in females (1, 21). Similar findings have been reported for zidovudine-TP (1, 25), tenofovir-DP (21), and carbovir-TP (11). No gender-related differences in intracellular 3TC-TP pharmacokinetics were observed in the present study ($P > 0.20$).

There were smaller differences in the pharmacokinetics of intracellular 3TC-TP between the two dosing regimens than in plasma. The intracellular 3TC-TP C_{24} and AUC_{24} were, on average, only 17 and 26% lower, respectively, with the 150-mg QD dose. One explanation is that saturation of the enzymatic conversion of 3TC-diphosphate (3TC-DP; the predominant anabolite and the rate-limiting step in the 3TC phosphorylation pathway) to 3TC-TP may occur at higher 3TC doses, resulting in pooling of the intermediate DP and continual persistence of the active TP over the course of the dosing interval, despite rapidly declining 3TC systemic concentrations (15).

Indeed, an apparent effect of treatment period upon 3TC-TP pharmacokinetics was observed. Higher 3TC-TP exposures were present at day 30 compared to day 10, suggesting there may be incomplete washout of the TP (or DP) anabolites during days 11 to 20 or changes to 3TC phosphorylation patterns over the duration of treatment. Sequestration inside the cell may also explain why there is little association between intracellular 3TC-TP and plasma 3TC concentrations. In the present study, the geometric mean elimination $t_{1/2}$ of 3TC-TP were 16.6 and 20.9 h, for the 300-mg and 150-mg QD doses, respectively. Moore et al. reported a 3TC-TP $t_{1/2}$ of ca. 15 to 16 h (based data derived from 12-h pharmacokinetic profiles, after 3TC 300-mg and 150-mg BID dosing) (18); however, these values could be seen as an underestimation of the "true" terminal elimination $t_{1/2}$ and persistence of the anabolite(s) beyond the dosing interval. Indeed, there are reports of up to a 3-fold accumulation of 3TC-TP over the course of 12 days (2).

In conclusion, these pharmacokinetic data show that 3TC at 150 mg is not bioequivalent to the standard regimen of 300 mg once daily. Although we do not know a definitive threshold of 3TC-TP to predict efficacy, and it cannot be ruled out that a lower dose may give a concentration on the plateau of the concentration effect curve, we consider that future evaluation of the lower dose is not warranted based on these data.

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